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(54) Title of the Invention: Method of manufacturing cold meat products of excellent storage characteristics

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### Specification

#### 1. Title of the invention

Method of manufacturing cold meat products of excellent storage characteristics

#### 2. Claims

1. Method of manufacturing cold meat products of excellent storage characteristics characterised in that fermented milk prepared using bifidus bacteria is added to the cold meat product.

2. Method of manufacturing cold meat products of excellent storage characteristics according to claim 1 wherein the bifidus bacteria employed in the preparation of the fermented milk that is added are one or a combination of two or more of: *Bifidobacterium longum*, *Bifidobacterium bifidum*, *Bifidobacterium adolescentis*, *Bifidobacterium infantis*, or *Bifidobacterium breve*.

3. Method of manufacturing cold meat products of excellent storage characteristics according to claim 1 wherein the addition ratio of the fermented milk is at least 100 g per kg of raw material meat.

4. Method of manufacturing cold meat products of excellent storage characteristics according to claim 1 wherein the content of residual lactose in the added fermented milk is no more than 0.5%.

#### 3. Detailed description of the invention

[Field of industrial application]

The present invention relates to a method of manufacturing cold meat products whereby multiplication of micro-organisms that cause problems in regard to hygiene of stored food products is suppressed and that provides excellent storage capability with scarcely any change of flavour or pH.

[Prior art]

Introduction of lactic acid bacteria into food is frequently carried out with the object of (1) improving storage characteristics, (2) improving flavour, or (3) health benefits.

Lactic acid bacteria have conventionally been employed also in cold meat products with the object of improving storage characteristics and improving flavour. For example *Lactobacillus plantarum*, *Pediococcus acidilactis* or *Pediococcus cerevisiae* are marketed as starters for the manufacture of fermented sausages, either on their own or in the form of mixtures (N. Yano: Dairy Farming Science. Studies of Food Products 30(6)A243 ('81)). The acid that is produced by the *L. plantarum* or *P. cerevisiae* added as a starter lowers the pH, thereby improving storage characteristics of the sausage, and confers a spicy flavour (J.L. Smith, S.A. Palumbo: J. of Food Prot., 44(12)936('81)).

In addition, published Japanese patent No. Sho. 59- 10787 discloses the manufacture of a sausage of excellent storage properties of pH 4.0 to 4.8 by the addition of about 10% of fermented solidified milk protein with respect to the meat constituting the raw material.

Also, in contrast to the case of fermented sausage, attempts have been made to improve storage characteristics by the direct addition of lactic acid bacteria starter to raw meat such as chicken, minced meat or steak or cold meat products such as meat products.

Such lactic acid bacteria include *Lactobacillus brevis*, *Pediococcus cerevisiae*, and *Lactobacillus plantarum* etc. (J.L. Smith, S.A. Palumbo: J. of Food Prot., 46(11)997('83)).

Apart from this, there are examples of the use of *Leuconostoc citrovorum*, and *Streptococcus diacetylactis* (A.L. Branen et al., J. of Food Sci., 40:446 ('75), examples of the use of *Lactobacillus bulgaricus* for yoghurt (S.E. Gilliland, M.L. Speck: J. of Food Sci., 40:903('75)), and examples of spraying *Streptococcus lactis* and *Leuconostoc citrovorum* on to both sides of steak, together with ascorbic acid (S.G. Reddy, M.L. Chen: J. of Food Sci., 40:314('75)).

[Problem that the invention is intended to solve]

Since a fermentation step enters into the process of manufacture of the aforementioned fermented sausage, acidity of course increases, with a fall in pH (to the vicinity of pH 5). Kato and co-workers have pointed out that one of the reasons why such fermented sausage is as yet scarcely familiar in Japan is that its acidity and peculiar flavour are not in accordance with Japanese taste (T. Kato et al.: Journal of agricultural chemistry of Japan, 59(1)11('85)).

Also, since most cold meat products are usually stored under aerobic conditions below 10 °C, practically all of the metabolic activity of the various types of lactic acid bacteria that are employed by being added to cold meat products in the prior art described above is maintained under such ordinary

The present invention consists in a method of manufacturing cold meat products of excellent storage characteristics characterised by the addition of bifidus bacteria fermented milk to the cold meat product.

The "cold meat products" referred to in the present invention are meat products such as minced meat, sliced meat, meat cuts, or processed meat such as uncooked sausage, uncooked hamburgers, uncooked "shumai"<sup>1</sup> or uncooked "gyoza"<sup>2</sup> which are distributed cold ie in unsterilised condition.

Examples that may be given of the bifidus bacteria which are employed in the preparation of fermented milk that is added to cold meat products according to the invention in order to confer storage characteristics include: the *Bifidobacterium longum* of human origin that are normally employed in food products (principally milk products), *Bifidobacterium bifidum*, *Bifidobacterium adolescentis*, *Bifidobacterium infantis*, and *Bifidobacterium breve*.

For the preparation of the bifidus bacteria fermented milk, the known method may be employed of for example inoculating bifidus bacteria starter into a defatted powdered milk reduced medium containing 10% to 20% of milk solids-not-fat (SNF) and culturing anaerobically for 16 hours to 24 hours at 35 to 40 °C. Preferably, however, preparation is conducted with the residual lactose content in the fermented milk adjusted to under 0.5%.

Although, if bifidus bacteria fermented milk is added to meat products, hygienic bacteria such as *Pseudomonas* and *Flavobacterium* present in the meat products are suppressed, contaminating lactic acid bacteria multiply during storage, causing a drop in pH; it is therefore preferable to make the residual lactose content in the bifidus bacteria fermented milk less than 0.5%.

#### Test example 1

The effects on flavour and drop in pH during storage of uncooked sausage to which had been respectively added bifidus bacteria fermented milk samples with contents of residual lactose varying from 0.1% to 2.0% after completion of fermentation of the bifidus bacteria fermented milk were investigated.

Uncooked sausages were manufactured by the same method as in the case of Embodiment 1 apart from the residual lactose content after completion of fermentation of bifidus bacteria fermented milk, and respectively stored at 15 °C.

The results were as shown in Table 1.

Table 1

Residual lactic acid in the fermented milk	Measurem ent items	Number of days of storage	

<sup>1</sup> a kind of dumpling stuffed with minced meat

<sup>2</sup> a kind of dumpling stuffed with minced meat

		Start	1 day	2 days	3 days	5 days	7 days
0.1%	pH flavour	5.80 Good	5.80 Good	5.78 Good	5.70 Good	5.64 Good	5.66 Rather poor
0.3 %	pH flavour	5.81 Good	5.80 Good	5.75 Good	5.71 Good	5.60 Good	5.61 Rather poor
0.5%	pH flavour	5.80 Good	5.79 Good	5.72 Good	5.61 Good	5.52 Good	5.55 Rather poor
0.75%	pH flavour	5.82 Good	5.76 Good	5.71 Good	5.53 Rather hard	5.20 Hard and sour	5.11 Hard and sour
1.0%	pH flavour	5.82 Good	5.76 Good	5.71 Good	5.53 Rather hard	5.20 Hard and sour	5.11 Hard and sour
2.0%	pH flavour	5.84 Good	5.77 Good	5.70 Good	5.52 Rather hard	5.06 Hard and sour	4.83 Hard and sour

It is found that in the case of uncooked sausage with residual lactose content of 0.75% or more lactic acid is produced by the third day which results in hardness that has an adverse effect on quality.

On the other hand, if the residual lactose content is under 0.5%, the flavour of the uncooked sausages is good for up to five days.

The ratio in which the bifidus bacteria fermented milk is to be added is preferably 100 g to 500 g, and even more preferably 100 g to 300 g to 1 kg of raw material meat. If less than 100 g are added, no improvement in storage characteristics of the cold meat product can be expected but, if more than 500 g are added, although the storage characteristics of the meat product are improved, the original flavour of the meat product is impaired, which is undesirable.

#### Test example 2

The effects on taste and number of live bacteria in storage of uncooked sausage were investigated, varying the added amount of bifidus bacteria fermented milk from 15 g to 1000 g per kg of pork.

The uncooked sausages were manufactured by the same method as in the case of Embodiment 1 apart from the ratio of added bifidus bacteria fermented milk with respect to the pork, and were stored at 15 °C in each case.

The results were as shown in Table 2.

storage conditions. Consequently, in cold meat products to which lactic acid bacteria have been added as starter in accordance with these prior art examples, acidity increases, albeit slowly, during storage and pH is lowered.

Consequently, even though multiplication of hygienic bacteria in the cold meat product is suppressed during refrigeration as described above, the pH falls and the flavour originating from lactic acid bacteria appears; this of course does not conform to the tastes of the Japanese, who are not traditionally a meat-eating race.

It is therefore highly desirable, in improving the storage characteristics of cold meat products, to achieve storage characteristics such that the pH does not vary. In order to prevent change of pH during storage, it suffices to deactivate the metabolic activity of the lactic acid bacteria, so even in the case of fermented milk where lactic acid bacteria in accordance with the prior art were employed, there was no possibility of increase of acidity and fall of pH during refrigeration if the lactic acid bacteria were sterilised beforehand by pre-heating sterilisation before this sterilised fermented milk was added to the meat product.

However, the mechanisms of the antibacterial action of lactic acid bacteria are thought to comprise the following five:

- (1) production of acid with lowering of pH
- (2) production of hydrogen peroxide
- (3) production of antibacterial substances
- (4) competition for nutrients
- (5) lowering of redox potential

(K. Shibazaki: Food Products and Low Temperature 7(1)5('81)), so if the fermented milk is sterilised by heating, the hydrogen peroxide is broken down, the antibacterial substances are deactivated, and the redox potential rises, with the result that there is a considerable drop in antibacterial action compared with unsterilised fermented milk.

An object of the present invention is to solve the above drawbacks and to provide a method of manufacturing cold meat products whereby storage characteristics can be improved without changes in pH or flavour.

[Means for solving the problem]

As a result of various studies concerning improvement in storage characteristics of cold meat products, the present inventors discovered that the above problem could be solved by the addition of fermented milk prepared using bifidus bacteria (hereinbelow called "bifidus bacteria fermented milk").

Specifically, they ascertained experimentally by studies over many years that, since the bifidus bacteria are anaerobic bacteria, they have no metabolic activity under the ordinary aerobic storage conditions of cold meat products, but they have an equivalent or better effect in suppressing hygienic bacteria which created a problem in refrigeration of cold meat products to the suppression effect possessed by the prior art lactic acid bacteria. They thereby perfected the present invention.

Table 2

Number of days storage *Added amount		Start	1 day	2 days	3 days	5 days	7 days
50 g	Number of live bacteria** Taste	1.65 X 10 <sup>5</sup> Good	1.81 X 10 <sup>5</sup> Good	2.32 X 10 <sup>5</sup> Good	4.8 X 10 <sup>5</sup> Good	1.26 X 10 <sup>6</sup> Rather poor	9.8 X 10 <sup>6</sup> Poor
100 g	Number of live bacteria Taste	9.3 X 10 <sup>4</sup> Good	9.1 X 10 <sup>4</sup> Good	1.12 X 10 <sup>5</sup> Good	1.16 X 10 <sup>5</sup> Good	4.8 X 10 <sup>5</sup> Good	4.6 X 10 <sup>5</sup> Rather poor
300 g	Number of live bacteria Taste	7.8 X 10 <sup>4</sup> Good	8.1 X 10 <sup>4</sup> Good	9.6 X 10 <sup>4</sup> Good	7.3 X 10 <sup>4</sup> Good	6.6 X 10 <sup>4</sup> Good	6.8 X 10 <sup>4</sup> Rather poor
500 g	Number of live bacteria Taste	7.2 X 10 <sup>4</sup> Good	6.8 X 10 <sup>4</sup> Good	6.1 X 10 <sup>4</sup> Good	5.6 X 10 <sup>4</sup> Good	4.7 X 10 <sup>4</sup> Rather poor	3.8 X 10 <sup>4</sup> Rather poor
700 g	Number of live bacteria Taste	6.8 X 10 <sup>4</sup> Excessive moisture	6.3 X 10 <sup>4</sup> Very sour	5.2 X 10 <sup>4</sup> Very sour	4.3 X 10 <sup>4</sup> Very sour	3.1 X 10 <sup>4</sup> Very sour	1.67 X 10 <sup>4</sup> Very sour
1000 g	Number of live bacteria Taste	6.5 X 10 <sup>4</sup> Excessive moisture	5.1 X 10 <sup>4</sup> Very sour	3.6 X 10 <sup>4</sup> Very sour	2.26 X 10 <sup>4</sup> Very sour	1.04 X 10 <sup>4</sup> Very sour	6.8 X 10 <sup>3</sup> Very sour

\* Added amount of fermented milk with respect to 1 kg of pork

\*\* Number of live bacteria determined by a CVT agar medium

When the added amount of bifidus bacteria fermented milk was 50 g per kg of pork, by the fifth day the flavour had become rather poor because of the increase in the number of live bacteria. Also when the

added amount of bifidus bacteria fermented milk was 700 g per kg of pork, although there was no increase in the number of live bacteria even after seven days, this was unsuitable because a strong sour taste was produced after two days.

#### Embodiment 1

1% of *Bifidobacterium longum* ATCC 15707 starter were inoculated into a defatted powdered milk reduced medium (SNF 10%) and cultured for 16 hours anaerobically by the steel wool method. In this case, neutral culturing was performed and bifidus bacteria fermented milk whose residual lactose content was adjusted to 0.4% was obtained on completion of the fermentation.

1 kg of pork that had been minced using a chopper ( $\phi$  4.8 mm) was finely minced for one minute using a silent cutter that had been cooled beforehand using dry ice and 6 g of phosphate, 10 g of onion, 17 g of common salt, 3 g of pepper powder and 200 g of the above bifidus bacteria fermented milk were then added thereto, and again minced and stirred for a further 1.5 minutes using the silent cutter. To this were added 200 g of pork back fat minced beforehand using a chopper ( $\phi$  4.8 mm) and the mixture was then finely minced and stirred for a further 3.5 minutes. Uncooked sausages were then manufactured by packing this into collagen casings manufactured by Nippy Inc.

#### Comparative Example 2

Fermented milk of lactose acidity 1% was obtained by inoculating a starter consisting of a mixture of equal amounts of *Streptococcus lactis* subsp. *diacetylactis* ATCC 11007 and *Leuconostoc cremoris* ATCC 19254 into a defatted powdered milk reduced culture medium (SNF 10%) and culturing for 20 hours at 32 °C.

Uncooked sausages were manufactured by the same method as in the case of Embodiment 1, apart from addition of the same quantity of the above fermented milk instead of the bifidus bacteria fermented milk.

The three types of sausage of Embodiment 1 and Comparative Examples 2 and 3 were stored at temperatures of 5, 10 and 15 °C and their flavour evaluated after simultaneously boiling. The results were as shown in Table 3.

In the case of Embodiment 1, a fully satisfactory bacteria suppressing effect was displayed and all the sausages were excellent after a storage time of five days, showing no change of pH or change of flavour.

In the case of Comparative Example 1, spoilage took place after three days at 10 °C and two days at 15 °C, respectively.

In the case of Comparative Example 2, although a bacteria suppression effect was displayed, the pH fell with storage and by five days at 10 °C or two days at 15 °C sourness was clearly discernible, lowering the product value.

Table 3. Results of storage test of uncooked sausage

Sample			Start time	Day 1	Day 2	Day 3	Day 5
	5 °C	pH Number of live bacteria*		6.1 $7 \times 10^4$	6.1 $7 \times 10^4$	6.1 $7 \times 10^4$	6.1 $7 \times 10^4$
	10 °C	pH Number of live bacteria	pH 6.1 $7 \times 10^4$ /g	6.1 $7 \times 10^4$	6.1 $7 \times 10^4$	6.0 $7 \times 10^4$	6.0 $7 \times 10^4$
	15 °C	pH Number of live bacteria		6.1 $7 \times 10^4$	6.0 $6 \times 10^4$	6.0 $6 \times 10^4$	5.9 $5 \times 10^4$
	5 °C	pH Number of live bacteria		6.2 $4 \times 10^5$	6.2 $3 \times 10^6$	6.2 $8 \times 10^6$	6.2 $2 \times 10^8$
	10 °C	pH Number of live bacteria	pH 6.2 $3 \times 10^5$ /g	6.2 $8 \times 10^5$	6.2 $1 \times 10^7$	6.2 spoiled	
	15 °C	pH Number of live bacteria		6.2 $5 \times 10^6$	6.1 spoiled		
	5 °C	pH Number of live bacteria		5.9 $3 \times 10^5$	5.8 $3 \times 10^5$	5.7 $2 \times 10^5$	5.5 $7 \times 10^4$
	10 °C	pH Number of live bacteria	pH 5.9 $3 \times 10^5$ /g	5.8 $2 \times 10^5$	5.6 $2 \times 10^5$	5.4 $7 \times 10^4$	5.1 $3 \times 10^4$
	15 °C	pH		5.6	5.1**	4.9**	4.7**



	Number of live bacteria		$1 \times 10^5$	$7 \times 10^4$	$4 \times 10^4$	$3 \times 10^4$
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\* Number of live bacteria determined by CVT agar medium.

\*\* Sourness clearly discernible.

#### Embodiment 2

0.5 % yeast extract was added to 10% defatted powdered milk medium and inoculated with 1% of *Bifidobacterium longum* ATCC 15707 and cultured for 16 hours at 37 °C by the steel wool method.

1.5 kg of pork minced using a chopper ( $\phi$  4.8 mm) were finely minced for one minute using a silent cutter that had been cooled beforehand using dry ice; 9 g of sunpolymer, 15 g of onion (finely minced), 25.5 g of common salt, 1.5 g of moguntia, 12 g of hens spice, 45 g of sodium casein, and 200 g of the aforementioned bifidus bacteria fermented milk were then added, and again finely minced and stirred for 1.5 minutes using a silent cutter. To this was then added 300 g of pork back fat minced beforehand using a chopper ( $\phi$  4.8 mm), and this was finely minced and stirred for a further 3.5 minutes. Next, uncooked sausages were manufactured by packing into collagen casings manufactured by Nippy Inc.

When the flavour was evaluated by boiling these uncooked sausages after storing for five days at 15 °C, the flavour was found to be excellent, just as it was immediately after manufacture, with no lowering of pH or spoilage smell.

#### [Benefit of the invention]

With this invention, storage characteristics of cold meat products can be improved without lowering of pH by adding fermented milk prepared using bifidus bacteria, which are anaerobic lactic acid bacteria, to the cold meat product.

Consequently, it offers the advantage that cold meat products can be provided that are in conformity with Japanese taste since the change of flavour produced by lowered pH resulting from addition of aerobic lactic acid bacteria as conventionally employed does not occur.

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